California Sea Grant College Program
Final Report

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The Role of Symbiotic Bacterial Metabolites in the Development of Toxic Phytoplankton Blooms

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Project Hypothesis

Most phytoplankton, including the toxic species of interest, cannot be grown axenically and require cohabitabing, associated, or symbiotic bacterial partners. However we have been able to prepare stable binary cultures of phytoplankton of interest with just a single species of bacteria (most of which are species of Marinobacter). While we recognize the culturable bacteria that we have isolated and are now studying are not necessarily qualitatively or quantitatively representative of the bacterial population associated with the phytoplankton in the wild, the ability to produce viable binary phytoplankton/bacterial cultures represents a critically important experimental tool. While other non-culturable bacteria could be quantitatively more important inhabitants of the "phycosphere" in the wild, our ability to ascertain the specific chemical determinants supplied by these bacteria and necessary to phytoplankton survival would be severely limited or impossible with such a heterogeneous and constantly changing assemblage. The ability to form stable, viable binary cultures indicates that the bacteria in question, irrespective of their numerical importance in a natural state, must provide some important factor for phytoplankton growth and greatly enhances our ability to define the nature of that substance and to test hypotheses based on it. Our hypothesis is that the critical element provided by the bacteria that control algal growth and survival involves siderophores, either directly through some form of iron trafficking between the species or indirectly possibly as boron-mediated interspecies cell-cell signaling molecules.

Project Goals and Objectives

The specific project objectives are:
1) To further explore the structure and iron binding characteristics of new siderophores produced both by members of the Marinobacter algicola clade of the gamma-proteobacteria and others closely associated with Gymnodinium catenatum, Alexandrium tamarense and other HAB-producing dinoflagellates as well as the ecologically more numerous alpha-proteobacteria.

2) To determine if the aforementioned phytoplankton in their vegetative state can utilize (transport) the iron from the siderophores produced by associated bacteria and thereby enhance their growth. There are several possible hypotheses: phytoplankton use siderophores produced by associated bacteria to directly to acquire iron. phytoplankton can acquire iron from these or other siderophores via an indirect route such as reduction. phytoplankton acquire iron from photoreactive siderophores either through their transient formation of Fe(II) or by uptake of the resulting newly decarboxylated Fe(III) siderophore complex.

3) To determine if the availability of iron derived in some way (vide supra) from their associated bacterial partners can trigger rapid outgrowth of dormant phytoplankton.

4) To use a combination of genomics and proteomics to help determine the other biological effects at the cellular / molecular level induced by siderophores produced by algal-associated bacteria - e.g. do siderophores, when bound to boron or iron, function as intra- (bacteria) or inter-(bacteria-dinoflagellate) species cell signaling, "quorum sensing" or regulatory molecules, which could affect items 1-3 above.

Briefly describe project methodology

To address the first objective, we have screened for and structurally characterized new phytoplankton-associated bacterial strains for novel siderophores. To address the second objective algal iron uptake from siderophore sources have been monitored by harvesting biomass at intervals during the growth phase and using 55Fe to

quantitatively measure uptake of iron directly and/or monitoring the rate of vegetative growth via chlorophyll flourecence. To address objective three we have generated a range of Marinobacter mutants deficient in siderophore production and regulation (using random transposon mutagenesis and/or chromosomal insertions from suicide vectors). Mutants are now being screened by bioassay for their effect on dinoflagellate cyst outgrowth, which is a measure of whether siderophore production is necessary to the bacterial-dinoflagellate symbiotic relationship. To undertake the final objective, we use real time PCR to examine siderophore, other iron regulated and housekeeping gene expression, and 2-D electrophoesis and MALDI-TOF to examine protein expression under a range of physiological conditions (e.g. trace boron/iron deprivation/excess; siderophore-complexed boron or iron).

Describe progress and accomplishments toward meeting goals and objectives.

We have isolated bacteria that are consistently found associated with phytoplankton cultures from different geographic locations and show their ability to produce an unusual siderophore that upon binding iron exhibits unprecedented photosensitivity. Subsequently, we have found that both the phytoplankton and their bacterial partners show a significant enhancement in iron uptake after photolysis suggesting that the associated bacteria may be "sharing" their newly bioavailable iron. The implications of our findings suggest a redefining of algal-bacterial interactions as a key component in phytoplankton nutrient acquisition that ultimately promotes algal growth whether it is primary productivity or harmful algal blooms. While these studies provided strong evidence for the involvement of specific clades of Marinobacter in enhancing iron uptake in dinoflagellates in laboratory cultures, the presence of these interactions in the environment remains circumstantial. In our more recent work we focused on the effects of light on gene expression related to iron uptake in Marinobacter clades responsible for VF production and examine their abundance in the North Atlantic, the Icelandic basin, and Antarctica. Our results illustrate a number of important points. First, pvsB (a surrogate for VF production) is readily detected and is widespread in all the environmental samples examined in this study. This is the first example of the identification of a siderophore biosynthetic gene in the marine environment that we are aware of. Second, all stations exhibit a consistent localization of pvsB genes near the surface with decreasing abundance with depth. The implication from this is that VF producing Marinobacter are concentrated in the photic zone, where sunlight will enhance the rate of photochemical production of Fe(III)' via photolysis of VF-Fe(III) chelate. Finally the correlation of pvsB abundance with chlorophyll a and its predominantly particle-associating ecology is consistent with the hypothesis that these Marinobacter spp. are likely to be algal “associated”.

PROJECT MODIFICATIONS:

Having shown that bacterial-algal interactions involving iron acquisition are important in the laboratory, we have moved into determining its ecological importance in the field. Here we are assesings the microbial diversity and abundance in the free-living vs. algal and particle associated bacterial communities associated with HAB events using several techniques. Typically we isolate particle- or algal-associated bacterial DNA for clone libraries. These have been collected from local water (SIO) as well as from cruises in which we have been able to participate. Eubacteria primers and taxa specific primers are being used to study diversity using the following approaches. Tyramide Signal Amplification-Fluorescent in situ Hybridization (TSA-FISH): Fluorescent in situ hybridization (FISH) is a powerful technique in addressing single cell ecology and uncultured microbial community structure. We have recently developed a successful FISH probe for detection of gamma-proteobacteria including Marinobacter DG893 in mixed culture with S. trochoidea. More specific probes will now be created for known algal associated, vibrioferrin-producing Marinobacter clades have also now been produced. Preliminary results are on environmental samples are encouraging.

Real-time Quantitative PCR – 5′-nuclease TaqMan assay: We utilize TaqMan assays in order to quantify abundance of particular groups associated with algal cells as compared to free-living bacteria. We hsve developed such TaqMan-based probes for the Marinobacter genus which we have already shown to be an important community member.

Siderophore biosynthesis related gene quantification and expression: In conjunction with studies of microbial diversity, we target siderophore biosynthetic pathways for gene quantification and expression studies. We have developed a real-time PCR SYBRGreen-based detection methods for vibrioferrin, aerobactin and petrobactin (all photoactive siderophores) biosynthetic genes. Degenerate primer pairs targeting all known open reading frames were designed. Preliminary results indicate that these genes are indeed present in surface waters and further work is ongoing to quantify their abundance and distribution in particle- (algal-) associated vs. free-living bacteria.

PROJECT OUTCOMES:

Our work is set apart from previous studies because it combines recent interests in bacterial-algal interactions, the role of iron in bloom formation, and bacterial mediated photochemical iron acquisition by algal cells into one

ecologically significant model. This model has now been tested in actual environmental samples obtained during several research cruises and several coastal HAB events.

IMPACTS OF PROJECT:
n/a

BENEFITS, COMMERCIALIZATION, AND APPLICATION OF PROJECT RESULTS:
n/a

ECONOMIC BENEFITS generated by discovery
None listed.

Issue-based forecast capabilities
The relationship we have uncovered between particular bacterial species, iron and bloom forming dinoflagellates opens the door for better forecasting of bloom events. In addition the ability to detect gene products for the "symbiotic" bacteria in the environment promises the possibility to detect potential bloom forming conditions early on.

Tools, technologies and information services developed
None listed.

Publications
Peer-reviewed journal articles or book chapters

Journal	BioMetals	**Issue Num**	25	**Page Num**	181-192	**Date**	2012
Title	Siderophore-Mediated Iron Uptake in two clades of Marinobacter spp. Associated with Phytoplankton: The Role of Light	**Authors**	Shady A. Amin, David H. Green, Astrid Gaerdes, Lyndsay Trimble, Ariel Romano, and Carl J. Carrano.				
Journal	J. Inorg. Biochem.	**Issue Num**	107(1)	**Page Num**	96-103	**Date**	2011
Title	The Fe(III) and Ga(III) coordination chemistry of Novel tetramic acid degradation products of homoserine lactone bacterial quorum sensing molecules.	**Authors**	Ariel A. Romano, Tobias Hahn, Nicole Davis, Colin Lowery, Kim D. Janda, Lars H. Boettger, Berthold F. Matzanke, and Carl J. Carrano				
Journal	BioMetals	**Issue Num**	25	**Page Num**	135-147	**Date**	2012
Title	Siderophore Mediated Iron Transport in the genus Marinobacter.	**Authors**	Shady A. Amin, David H. Green, Dhuha Al Waheeb, Astrid Gaerdes, and Carl J. Carrano.				

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INTERNATIONAL IMPLICATIONS
This is a joint project between SDSU and the Scottish Association for Marine Science in Oban, Scotland.
Drs. F.C. Kuepper and D.H. Green are co-PIs. We have had several student and faculty exchanges between our two labs giving the students a great international experience.

FOR ALL STUDENTS SUPPORTED BY THIS GRANT, PLEASE LIST:

Volunteer Count 5

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